1.0 SCIENTIFIC ABSTRACT OF THE PROTOCOL

Cystic fibrosis (CF), the most common lethal genetic disease in North America, is caused by defects in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. The CFTR gene product is required for regulation of epithelial chloride transport in multiple organs, including the airways. CF lung disease develops progressively as abnormally viscous secretions that lead to airway obstruction, infection, inflammation, and fibrosis. It ultimately may lead to respiratory failure, which is the cause of death in greater than 90% of CF patients. It is thought that correction of the underlying CFTR gene defect may result in therapeutic effect on the progressive lung disease.

The vector (tgAAVCF) is derived from adeno-associated virus (AAV), which is not known to cause human disease. This transducing vector can be generated at sufficiently high titers that it is practical as a delivery system and the vector preparation can be purified to near homogeneity, removing contaminants and process materials.

Furthermore, AAV-CFTR vectors have been shown to transduce and express recombinant CFTR *in vivo* after delivery to the airway surface of animals. Long-term vector expression, up to 6 months after a single-dose administration, has been observed in the New Zealand white rabbit and rhesus monkey models. Administration of higher doses of tgAAVCF by aerosol inhalation to rhesus macaques has demonstrated dose-dependent gene transfer and gene expression. Repeat dose administrations at these higher doses were well tolerated, and dose dependent gene transfer was observed. In some animals, serum neutralizing antibody titers to AAV2 increased and low titers of antibody and an elevation of lymphocytes were observed in bronchial wash fluid. There was no other clinical pathology or histologic findings related to the exposure of vector. Biodistribution of the vector to the gonads was not observed.

Ninety cystic fibrosis patients have been administered single and multiple doses of tgAAVCF with no serious adverse events attributed to this vector. Data from

administration to the maxillary sinus, nose, lung lobe, and whole lung have shown a dose dependent, persistent gene transfer following single dose administration. A number of functional measurements, including sinus potential difference measurements and changes in IL-8 levels, are suggestive of biological activity.

In a recently completed randomized, placebo-controlled trial, subjects ≥12 years of age with cystic fibrosis and mild lung disease were randomized to three aerosolized doses of either $1x10^{13}$ DRP of tgAAVCF or placebo at 30-day intervals. No differences in safety or tolerability were observed between the 20 subjects randomized to tgAAVCF, and the 17 subjects randomized to placebo. Trends in improvement in pulmonary function tests and IL-8 levels in induced sputum were observed. At Day 30, the mean FEV_1 increased 0.10 ± 0.20 L in subjects randomized to tgAAVCF, whereas it decreased 0.04 ± 0.18 L in subjects randomized to placebo (p=0.04). At Day 14, sputum IL-8 levels decreased 0.09 ± 0.19 $\log_{10}$ ng/mL in subjects randomized to tgAAVCF, whereas it increased 0.12 ± 0.27 $\log_{10}$ ng/mL in subjects randomized to placebo (p=0.03).

This study is designed to confirm the encouraging trends in pulmonary function and IL-8 levels observed in the previous trial. A total of 100 subjects ≥12 years of age with confirmed CF and mild lung disease (FEV_1 ≥ 60% predicted) will be randomized to receive two administrations of either tgAAVCF at a dose of $1x10^{13}$ DRP or placebo delivered via aerosol 30 days apart. The primary endpoint will be pulmonary function, as assessed by spirometry, and measured by FEV_1. Secondary endpoints include biologic markers in induced sputum, intravenous antibiotic use for CF pulmonary exacerbations, and safety, as assessed by adverse event reporting, clinical examination and laboratory monitoring. The sample size of 50 subjects per treatment group will provide 93% power to detect the 30-day change in FEV_1 seen in the previous trial as, well as adequate power for detecting differences in IL-8 between treatment groups. The study will be overseen by the Cystic Fibrosis Foundation Data Safety Monitoring Board, who will be made aware of serious adverse events as they occur, and review unblinded data at regular intervals.